Functional Analysis of HIV-2 Vpx Protein −Multiple Roles of the Poly-proline Motif

Halil Ibrahim Ciftci

Department of Bioorganic Medicinal Chemistry, Medicinal Chemistry Course, Major in Drug Development, School of Pharmacy

HIV-1 and HIV-2 have some accessory proteins, and some of them have been revealed to carry functions as arms to degrade host anti-viral factors. One of them is Vpx carried by HIV-2. Vpx is critical for reverse transcription of the viral RNA genome in macrophages. Its mechanism is that Vpx becomes adapter protein, forms Cul4-DDB1-DCAF1 E3 ligase complex and degrades the host restriction factor SAMHD1 by proteasome. Furthermore, it was suggested that Vpx is important in activated T lymphocytes by SAMHD1 degradation-independent mechanism. In this study, we first tried to determine whether the mechanism which confers the capacity for viral replication in macrophages is solely dependent on its ability to degrade SAMHD1.

1. Mutational Analysis of Vpx Protein

We investigated the ability of a series of Vpx mutants carrying 19 point mutations in its scattered regions (Figure) to degrade SAMHD1 in human 293T cells and found that large amount of Vpx prevents the degradation. Then, effects of these mutants were compared with abilities to confer viral infectivity. As results, relatively good correlation was identified between these two characteristics. There were a few exceptions. Among them, C87A (a mutant carrying A instead of the 87th C known to coordinate with zinc) and P109A (a mutant in the C-terminal poly-proline motif (PPM)) were candidates for mutants lacking the SAMHD1-degradation independent function of Vpx.

2. Role of PPM for Oligomerization of Vpx

Vpx amount dependency on SAMHD1 degradation was examined, and it was found that large amount of P109A mutant degrades SAMHD1 efficiently unlike WT Vpx.

Another PPM mutant P106A also shows the same character. One plausible explanation is that Vpx multimerization at high concentration interferes with formation of the Cul4-DDB1-DCAF1 E3 ligase complex, however, P106A and P109A do not oligomerize well. We confirmed that these mutants were clearly deficient in Vpx-Vpx interactions compared with those observed for the WT and the other mutants of Vpx.

3. Role of PPM for Specific Degradation of Vpx in Macrophages

C87A and P109A mutants were analyzed in detail. We found that C87A has low expression level in a context of full-genomic clone. P109A mutant of Vpx was incorporated into virions similar to its wild-type. The P109A was defective at reverse transcription stage. Unexpectedly, endogeneous SAMHD1 was degraded by P109A mutants in human macrophages or macrophages-like cells, but the degradation was less effective than in the cells infected with wild-type virus. Among the other alanine mutants of PPM, P103A degraded SAMHD1 like wild-type, but the ability of P104A-P108A to degrade SAMHD1 was not enough in the cells.

This and previous study suggest that the PPM of Vpx has multiple functions, degradation of SAMHD1 in macrophages, and facilitating translation of Vpx (previous result). Furthermore, PPM has ability to oligomerize Vpx, regulating SAMHD1 degradation negatively in the presence of high levels of Vpx. In this way, the amount of Vpx incorporated into virions may be adjusted to be appropriate for the degradation. This study would give new information on not only battle between host anti-viral factors and accessory proteins, but also unique protein region, PPM.

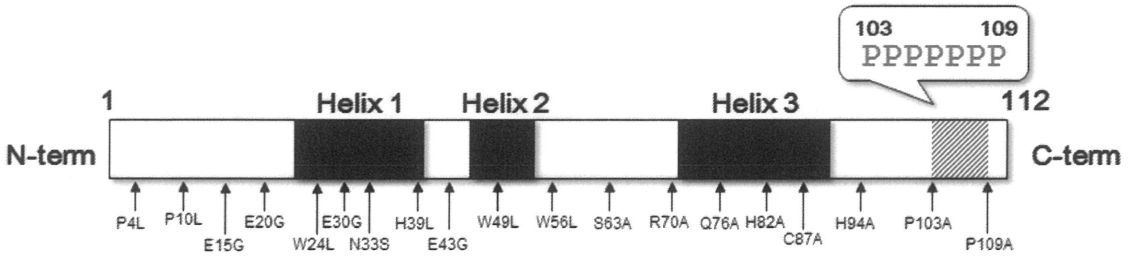

Figure. Structure of Vpx and positions of the introduced mutations.